DP Barcode: D422896 MRID No.: 49463701

DATA EVALUATION RECORD
72-2 -- ACUTE LC_{50} TEST WITH A FRESHWATER INVERTEBRATE
OCSPP 850.1020

1. **CHEMICAL:** Gamma-cyhalothrin PC Code No.: 128807

2. **TEST MATERIAL:** Gamma-cyhalothrin Purity: 97.9%

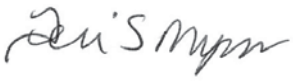
3. **CITATION**

Authors:	Bradley MJ
Title:	Gamma-Cyhalothrin- Acute Toxicity to Freshwater Amphipods (*Hyalella azteca*) Under Flow-Through Conditions
Study Completion Date:	August 11, 2014
Laboratory:	Smithers Viscient Wareham, Massachusetts
Sponsor:	Pyrethroid Working Group FMC Corporation Ewing, New Jersey
Laboratory Report ID:	13656.6184
MRID No.:	49463701
DP Barcode:	D422896

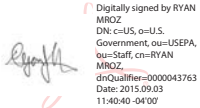
4. **REVIEWED BY:** John Marton, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 04/22/15

APPROVED BY: Teri S. Myers, Ph.D., Environmental Scientist, CDM Smith

Signature:  **Date:** 05/13/15

5. **APPROVED BY:** Ryan Mroz, Biologist, OPP/EFED/ERB-1

Signature: 

Date:

6. **DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-

case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Scientific Name of Test Organism:	*Hyalella azteca*
Age of Test Organism:	10 days old
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean measured

8. CONCLUSIONS:

A range finding at nominal concentrations of 0.050, 0.10, 0.20, 0.40 and 0.80 ng/L produced 35, 15, 20, 80 and 95% mortality respectively. After 96 hours of exposure in the definitive test, mortality was 5% in the negative control, and solvent control. Mortality was 50, 80, 95, 100 and 100% in the mean-measured, 0.083, 0.13, 0.22, and 1.5 ng ai/L treatment levels, respectively. Immobility was observed in all treatment groups. There was at least a 50% drop in measured concentrations at the 0 and 96 hour analytical measurements in all but the highest treatment group (which was 42%). The 96-hr EC50 (with 95% C.I.) value was 0.0823 (0.0462 to 0.103) ng ai/L, based on mean-measured concentrations.

Results Synopsis

96-hour LC_{50}: 0.0823 ng ai/L 95% C.I.: 0.0462-0.103 ng ai/L
Probit Slope: 4.02 95% C.I.: 1.66-6.38
NOAEC (visually determined): < 0.08 ng ai/L
Sublethal effects: Immobilization

Endpoint(s) affected: Survival and sub-lethal effects

9. ADEQUACY OF THE STUDY

A. Classification: This study **is scientifically sound** and is classified as **/supplemental (quantitative)**

B. Rationale: There were no test concentrations producing <50% mortality (50% in the lowest). However, without measured results from the range finding test the concentrations

selected for the definitive were not unreasonable. There was also at least a 50% drop in test concentration from 0 hour to the 96 hour measurement across all but the highest test concentrations (42%). Given the nature of the chemical, the low test concentrations (measured right up to the LOQ = 0.053 ng ai/L), and response viewed the reviewer does not believe additional testing would produce a much different endpoint. Consequently, this study may be used quantitatively in risk assessment, taking into account the uncertainties mentioned above.

C. Reparability: N/A

10. Guideline Deviations: This study was conducted following a protocol that generally meets the testing requirements of the U.S. EPA's Ecological Effects Test Guideline (Draft) OCSPP 850.1020 Gammarid Acute Toxicity Test; and the U.S. EPA's Ecological Effects Test Guideline (Draft) OCSPP 850.1000 Special Considerations for Conducting Aquatic Laboratory Studies. The following deviations from OCSPP 850.1020 were noted:

1. *Hyalella azteca* is not one of the preferred non-daphnid test organisms.
2. The instar of the test organisms was not specified.
3. Test organisms were fed during the definitive test.
4. Recommended temperature during the definitive test (20-24°C) exceeded the recommended temperature of 18±1°C. However, these recommendations are based on the genus *Gammarus*.
5. Biomass loading rate was not specified. However, only 10 amphipods were in each test vessel (1.8 L fill volume).
6. There was at least a 50% drop in measured concentrations in all but the highest treatment level.
7. There was no treatment concentration with less than 50% mortality.

Deviations 6 and 7 impact the acceptability of the study.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the effects of gamma-cyhalothrin to *Hyalella azteca* following acute exposure for the purpose of chemical re-registration.

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Species Preferred species is *Daphnia magna*	*Hyalella azteca*
All organisms are approximately the same size and weight?	Yes
Life Stage Daphnids: 1st instar (<24 h). Amphipods, stoneflies, and mayflies: 2nd instar. Midges: 2nd & 3th instar.	10 days old
Supplier	Laboratory cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 7 days	Continuous for main culture, 10 days for amphipods used in definitive test. Amphipods were obtained by isolating sexually mature amphipods from the main culture 11 days prior to test initiation. On the day following adult isolation, young produced by these organisms were pipetted into 1.0-L beakers containing 0.80 L of aerated water, where they were held until test initiation.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None reported
If treated for disease, was there no sign of the disease remaining during the 48 hours	N/A

Guideline Criteria	Reported Information
prior to testing?	
Feeding No feeding during the study.	During the holding period, the amphipods were fed a combination of yeast, cereal leaves, and flaked food suspension (YCT). During the definitive exposure, each replicate test vessel received 1.0 mL of YCT daily.
Pretest Mortality No more than 3% mortality 48 hours prior to testing.	None reported

C. Test System:

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water.	Laboratory well water. *H. azteca* are cultured in water from the same source as the dilution water used in the study and have successfully survived and reproduced over multiple generations.
Does water support test animals without observable signs of stress?	Yes
Water Temperature Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20-24°C The 20°C measurement was an isolated incident between 72 and 96 hours. The continuous measurements were within the range of 23 ± 1°C during this time period.
pH Prefer 7.2 to 7.6.	7.1-7.3

Guideline Criteria	Reported Information
Dissolved Oxygen Static: ∃ 60% during 1st 48 h and ∃ 40% during 2nd 48 h, flow-through: ∃ 60%.	6.7-9.4 mg/L (>75% of saturation) The lowest DO reading (6.7 mg/L) was measured in the nominal 1.6 ng ai/L treatment level at 96 hours.
Total Hardness Prefer 40 to 48 mg/L as $CaCO_3$.	64-68 mg/L as $CaCO_3$ Alkalinity: 18-20 mg/L as $CaCO_3$ Conductivity: 400-420 µS/cm
Test Aquaria 1. Material: Glass or stainless steel. 2. Size: 250 ml (daphnids and midges) or 3.9 L (1 gal). 3. Fill volume: 200 ml (daphnids and midges) or 2-3 L.	1. Glass beaker 2. 2 L 3. 1.8 L (depth of 14.5 cm) Each test vessel had a slot cut below the top edge of the beaker which was covered with 40-mesh NITEX® screen, adhered with silicone, for drainage. Each test vessel also contained a 3 cm^2 piece of 250-µm stainless steel mesh as a substrate.
Type of Dilution System Must provide reproducible supply of toxicant.	Intermittent-flow proportional diluter.
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	~10 vol/24 hours (90% replacement time of approximately 5 hours) System was calibrated prior to use and was confirmed at test termination. Diluter system function was monitored daily and a visual check of the system's operation was performed twice daily.

Guideline Criteria	Reported Information
Biomass Loading Rate Static: # 0.8 g/L at # 17°C, # 0.5 g/L at > 17°C; flow-through: # 1 g/L/day.	Not reported. However, DO levels were adequate throughout the definitive exposure.
Photoperiod 16 hours light, 8 hours dark.	16L:8D with 15-30-minute transition periods of low-light intensity. Light intensity ranged from 230-270 lux.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.	Acetone (0.050 mL/L)

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L, then no definitive test is required.	A 96-hour range-finding study was conducted using nominal concentrations of 0 (negative and solvent controls), 0.050, 0.10, 0.20, 0.40, and 0.80 ng ai/L with 20 amphipods per level (2 reps w/10 each). After 96 hours of exposure mortality was 5 and 11% in the negative and solvent controls, respectively, and 35, 15, 20, 80, and 95% in the 0.050, 0.10, 0.20, 0.40, and 0.80 ng ai/L groups, respectively. Immobilization was observed among surviving amphipods in all but the lowest treatment level.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	0.10, 0.20, 0.40, 0.80, and 1.6 ng ai/L

Guideline Criteria	Reported Information
Number of Test Organisms Minimum 20/level, may be divided among containers.	20/level, with 10 amphipods in each of two replicates
Test organisms randomly or impartially assigned to test vessels?	Yes
Water Parameter Measurements 1. Temperature Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	1. Measured in replicate A of all treatment levels at test initiation, in alternating replicates daily thereafter, and in all test chambers at test termination. Temperature was also continuously monitored in replicate B of the nominal 1.6 ng ai/L treatment level. 2. Dissolved oxygen and pH were measured in replicate A of all treatment levels at test initiation and in alternating replicates daily thereafter, and in all test chambers at test termination.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples were collected from each control and treatment level at 0 and 96 hours.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information

Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice Regulations (40 CFR, Part 160) with the following exceptions: routine water and food contaminant screening analyses were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts.
Control Mortality Static: ≤10% Flow-through: ≤5%	Negative Control: 5% Solvent Control: 5%
Percent Recovery of Chemical	64-94% of nominal based on mean-measured concentrations. QC spikes yielded recoveries ranging from 77.4 to 96.5% of nominal.
Raw data included?	Yes

Mortality

Concentration (ng ai/L)		Number of Organisms	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<LOQ	20	0	0	0	1
Solvent Control	<LOQ	20	0	0	0	1
0.10	0.083	20	4	6	7	10
0.20	0.13	20	1	6	8	16

0.40	0.22	20	2	9	13	19
0.80	0.49	20	8	15	17	20
1.6	1.5	20	5	10	15	20

Other Significant Results: No sub-lethal effects were observed in the controls throughout the 96-hour exposure period. Several surviving amphipods throughout the treatment groups were observed to be immobilized, with immobilization observed at all observation periods.

B. Statistical Results

Method: The 96-hour LC_{50} and associated 95% C.I. were estimated using the Trimmed Spearman-Kärber method via CETIS statistical software version 1.8. Mean-measured concentrations were used in the analysis.

96-hour LC_{50}: 0.086 ng ai/L 95% C.I.: 0.064-0.12 ng ai/L
Probit Slope: N/A 95% C.I.: N/A

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Trimmed Spearman-Kärber LC_{50} (95% C.I.)	0.0862 (0.0644-0.115) ng ai/L
Probit LC_{50} (95% C.I.)	0.0823 (0.0462-0.103) ng ai/L
Probit Slope (95% C.I.)	4.02 (1.66-6.38)

The reviewer analyzed mortality data using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Treatment data were compared to the negative control only. Results from the Trimmed Spearman-Kärber method were also reported. Toxicity values were based on the reported mean-measured concentrations.

15. REVIEWER'S COMMENTS:

The reviewer's results were based on the probit analysis whereas the study author reported results from the Trimmed Spearman-Kärber method. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

The TOC concentration for the dilution water source was 0.61 mg/L for March 2014.

Results from the periodic screening analysis of the dilution water were not provided. However, the study author reported that no pesticides, PCBs, or toxic metals were detected at concentrations that are considered toxic in any of the water samples analyzed in agreement with ASTM (2002) standard practices.

The in-life portion of the definitive toxicity test was conducted from March 10 to 14, 2014.

This study **is scientifically sound;** however, due to some minor guideline deviations it is classified as **supplemental (quantitative).**

16. REFERENCES:

Ives M. 2013. Comprehensive Environmental Toxicity Information System™, User's Guide. Tidepool Scientific Software, McKinleyville, California.

Mount DI, Brungs WA. 1967. A simplified dosing apparatus for fish toxicity studies. *Water Research* 1:20-29.

Sprague JB. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. *Water Research* 3:793-821.

CETIS Summary Report

OPPTS.850.1020 Gammarid Acute Toxicity **Smithers Viscient**

Batch ID:	10-5469-1053	**Test Type:**	Mortality (96-h)	**Analyst:**	
Start Date:	10 Mar-14	**Protocol:**	OPPTS.850.1020 Gammarid Acute Toxicity	**Diluent:**	Well Water
Ending Date:		**Species:**	Hyalella azteca	**Brine:**	Not Applicable
Duration:	NA	**Source:**	Lab In-House Culture	**Age:**	10dy
Sample ID:	20-3396-1417	**Code:**	128807 49463701	**Client:**	CDM Smith
Sample Date:	10 Mar-14	**Material:**	gamma-cyhalothrin	**Project:**	Insecticide
Receive Date:		**Source:**	Pyrethroid Working Group		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 128807 MRID 49463701
Sample Note: PC Code 128807 MRID 49463701

Point Estimate Summary

Analysis ID	Endpoint	Level	ng ai/L	95% LCL	95% UCL	TU	Method
14-3431-1542	96h Mortality Rate	LC5	0.0321	0.00508	0.0531		Linear Regression (MLE)
		LC10	0.0395	0.00836	0.0609		
		LC15	0.0455	0.0117	0.0668		
		LC20	0.0509	0.0152	0.0721		
		LC25	0.056	0.0191	0.077		
		LC40	0.0712	0.0334	0.0917		
		LC50	0.0823	0.0462	0.103		
20-4003-0313	96h Mortality Rate	LC50	0.0862	0.0644	0.115		Trimmed Spearman-Kärber

96h Mortality Rate Summary

C-ng ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	0.0%
0	Negative Control	2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	0.0%
0.083		2	0.5	0.5	0.5	0.5	0.5	0	0	0.0%	47.4%
0.13		2	0.8	0.8	0.8	0.8	0.8	0	0	0.0%	78.9%
0.22		2	0.95	0.315	1	0.9	1	0.05	0.0707	7.44%	94.7%
0.49		2	1	1	1	1	1	0	0	0.0%	100.0%
1.5		2	1	1	1	1	1	0	0	0.0%	100.0%

96h Mortality Rate Detail

C-ng ai/L	Control Type	Rep 1	Rep 2
0	Solvent Blank	0	0.1
0	Negative Control	0.1	0
0.083		0.5	0.5
0.13		0.8	0.8
0.22		1	0.9
0.49		1	1
1.5		1	1